STUDIES ON THE ECOLOGY, TAXONOMY AND PHYSIOLOGY OF SWEDISH INSECT-ASSOCIATED BLUE STAIN FUNGI, ESPECIALLY THE GENUS CERATOCYSTIS

BY

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ODENSE 1960
ANDELSBOGTRYKKERIET I ODENSE

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INAUGURAL DISSERTATION

BY

AINO MATHIESEN-KÄÄRIK

FIL. LIC., NORRL.

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INTRODUCTION

This paper is a summary of the following communications by the author:

1. MATHIESEN, A., 1950: Über einige mit Borkenkäfer assoziierte Bläuepilze in Schweden. – Oikos *2*,2: 275–308.
2. – 1951: Einige neue Ophiostoma-Arten in Schweden. – Sv. Bot. Tidskr. *45*: 203–232.
3. MATHIESEN-KÄÄRIK, A., 1953: Eine Übersicht über die gewöhnlichsten mit Borkenkäfern assoziierten Bläuepilze in Schweden und einige für Schweden neue Bläuepilze. – Medd. Statens Skogsforskningsinst. *43*,4: 1–74.
4. – 1960: A contribution to the physiology of Ophiostoma and some other blueing fungi. Growth and sporulation on synthetic media. – Symb. Bot. Upsal. *17*,1.

PREFACE

The investigations summarised in the present paper have been carried out in the Mycological Laboratory of the Forest Research Institute, Stockholm. I wish to express my sincere gratitude to the Director of the Mycological Section, Docent Erik Rennerfelt, who has introduced me to the problems of this research, has always helped with research material and has shown his interest during many stimulating discussions.

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Stockholm, Forest Research Institute,
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STUDIES ON THE ECOLOGY, TAXONOMY AND PHYSIOLOGY of Swedish Insect-associated blue stain Fungi, especially the Genus *Ceratocystis*

BY

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Aims of the Investigation and a Review of Previous Investigations on the blueing Fungi

The blueing or blue stain fungi are fungi, belonging to several different taxonomic groups, which have a dark mycelium and usually grow saprophytically in the wood of dead or dying standing trees or in timber causing a greenish or blueish-grey discolouration of the wood. They include a number of species of considerable economic and ecological interest. These fungi live on nutritive substances present in the cells, especially in the medullary rays and other parenchymatous cells; they attack lignified cell walls only to a limited extent but in the ray cells they may cause considerable destruction. The blueing fungi attack standing trees when their moisture content is low and timber at different stages of storage before it is completely seasoned. They spread very quickly both radially and longitudinally and thus can cause rapid discolouration and considerable economic losses. The greatest losses which are very difficult to control are those caused by blueing fungi which attack newly felled timber in the forest simultaneously with the infestations of bark beetles. These fungi, which mostly belong to the genus *Ceratocystis* (*Ophiostoma*) are spread by the insects and have a very interesting ecology. With their insect vectors they often form more or less firm associations that have been characterised by LEACH (1940) as true mutualistic symbiosis. Both the fungi and the bark beetles can exist and develop fully without their associates, but they are always found together in nature. Although many fungi may be found in the blue-stained wood of insect-infected trees, there are usually one or two species that are constantly found in wood which has just been invaded by a particular bark beetle. These associations may be very specific but no clue has hitherto been found to explain the basis of this

specificity. Other blueing fungi, frequently also of the genus *Ceratocystis*, invade the log when the bark has dried and begins to crack and peel off while still other blueing fungi predominate in timber yards on boards piled for seasoning.

Studies on the Swedish blue stain spreading insects and their fungal associates that have been carried out in the Entomological and Botanical Departments of the Forest Research Institute, Stockholm, presented an opportunity to investigate fungal material collected from different parts of Sweden. These investigations have been described in previous papers (MATHIESEN 1950 b, 1951, MATHIESEN-KÄÄRIK 1953). As little was known about the physiology of these ecologically interesting fungi, some physiological researches were later carried out partly with the ecologically restricted species and partly with some common and widely distributed blueing fungi (MATHIESEN 1950 a, MATHIESEN-KÄÄRIK 1960 a). The present paper summarises the results of these investigations up to the present time.

The insect-associated blueing fungi, especially the *Ceratocystis* species, produce several different types of spores, some of which are especially adapted for dispersal by insects. The existence of firm associations between blueing fungi and bark beetles must be closely connected with the dispersal biology of the fungi, which in this respect are entirely dependent on their insect vectors. Therefore it seemed that an investigation of some factors controlling the production of different types of spores and the viability of these spores would be of interest.

Previous ecological investigations on the occurrence of the blueing fungi, their distribution and their relationship to the bark beetles have been extensively reviewed by LEACH (1940) and MATHIESEN-KÄÄRIK (1953). In brief the results are as follows. Quite early (HARTIG 1878, VON SCHRENK 1903) attention was attracted to the simultaneous occurrence of blue stain and insect infestations; blue stain was thought to be caused by a specific fungus, *Ceratostomella pilifera*. MÜNCH (1907) showed that the blue stain was caused by several species of fungi, RUMBOLD (1931, 1936, 1941), and GROSMANN (1930, 1932) showed that different bark beetles had specific fungal associates and that the spores of the blue stain fungi were carried by the bark beetles both externally and internally. The experiments of NELSON & BEAL (1929), LEACH *et al.* (1934) and VERRALL (1939, 1941) showed that the blueing fungi could enter newly felled logs only with the aid of the insects and also that the fungi attacking the logs at later stages and the fungi causing blue stain in timber yards were different from these associated with insect infestations. WRIGHT (1935, 1938) and LEACH (1940) analysed more closely the relationship between bark beetles and blueing fungi and found that it appeared to be a case of true symbiosis. Later these relationships were investigated in several other countries, in Sweden

by RENNERFELT (1950), in England by BAKSHI (1951 a, b), in Poland by SIEMASZKO (1939) and in Japan by NISIKADO & YAMAUTI (1933) and others. Recently, FRANCKE-GROSMANN (1956) has made some interesting contributions to the ecology of the blueing fungi and has shown that the spores of one blue stain fungus, *Trichosporium tingens*, which is probably utilised as an ambrosia fungus by the beetles, are carried externally in the integumental glands where the spores can hibernate and even propagate in a yeast-like way. However no *Ceratocystis* spores were found in the glands.

The ecology of the closely related pathogenic fungi, *C. ulmi* and *C. fagacearum*, has been studied very thoroughly by a number of authors, including BUISMAN (1932), COLLINS (1936), SMITH (1936), HOLMES (1955), BECKMAN *et al.* (1953) and others.

Almost every ecological investigation on the blue stain fungi has resulted in the discovery and description of new species. At first the blue stain was thought to be caused by a single fungus, *Ceratostomella pilifera* (Fr.) Wint. but MÜNCH (1907) and HEDGCOCK (1906) discovered that several different fungi could produce this discolouration in wood and especially since about 1930 a large number of species of blueing fungi of *Ceratocystis* and other genera have been described. The rather confused history of the genera *Ceratocystis*, *Ophiostoma* and *Ceratostomella* has been thoroughly reviewed by RUMBOLD (1911), NANNFELDT (MELIN & NANNFELDT 1934) and HUNT (1956). Briefly, the genus *Ceratocystis* was established by ELLIS & HALSTED (in HALSTED 1890) and the description of the type species of the genus, *C. fimbriata*, was given by HALSTED & FAIRCHILD (1891). SACCARDO (1892) discarded *Ceratocystis* and transferred the species *C. fimbriata* to *Sphaeronema*; ELLIOTT (1923) later transferred it to *Ceratostomella*. As *Ceratostomella* Sacc. contained very different elements, NANNFELDT (MELIN & NANNFELDT 1934) transferred the species with irregular deliquescent asci to *Ophiostoma* H. & P. Syd. Until very recently however the generic name *Ceratostomella* has been used in America although *Ophiostoma* has been generally used in Europe. The genera *Endoconidiophora* and *Grosmannia* which were established for species with aberrant conidial stages, were also recognized by several authors. BAKSHI revived the generic name *Ceratocystis* in 1950 and transferred a number of *Ophiostoma* and *Ceratostomella* species to this genus. HUNT (1956) in his monograph on this genus recognised *Ceratocystis* as the valid name and regarded *Ophiostoma* as a later synonym for it. He transferred the *Ceratostomella* Sacc. species with evanescent asci to *Ceratocystis* and restricted *Ceratostomella* Sacc. to the species with regularly arranged persistent asci. He did not accept the division of the genus according to the different imperfect stages and regarded the genera *Endoconidiphora* Münch and *Grosmannia* G. Goid as synonyms of *Ceratocystis*.

A large number of taxonomic papers dealing with *Ceratocystis* and other blueing fungi have been published, for instance in Sweden by LAGERBERG, LUNDBERG & MELIN (1927) and MELIN & NANNFELDT (1932) and in other European countries by v. ARX (1952), BAKSHI (1950, 1951 a, b), GEORGEWITCH (1927), GOIDANICH (1935, 1936 a, b), GROSMANN (1930, 1932), LOOS (1932), ROBAK (1932), SIEMASZKO (1939), VARITCHAK (1931) and ZACH (1929). In America, among numerous taxonomic papers dealing with *Ceratocystis* may be mentioned those by ANDRUS & HARTER (1933), BLISS (1941), BRAMBLE & HOLST (1940), BRETZ (1952), DADE (1929), DAVIDSON (1935, 1942, 1944, 1953), ELLIOTT (1923), LEACH *et al.* (1940), LIMBER (1950), RUMBOLD (1929, 1931, 1936, 1941) and SHAW & HUBERT (1952), in addition there are other papers on some single species. There have also appeared numerous papers by NISIKADO & YAMAUTI (1933, 1934, 1935) and others in Japan. In Australia WEBB (1945) has contributed to the taxonomy of the blueing fungi. HUNT (1956) in his monograph carried out for the first time a critical examination of all the existing *Ceratocystis* species using both cultures and herbarium material. He showed that a number of different species described from different countries were synonymous and was able to reduce the number of species somewhat. His monograph deals with 63 species of which 39 were recognised.

From the papers listed above it is evident that among the blueing fungi attacking newly felled timber in the forest in conjunction with insect infestations, *Ceratocystis* species are predominant in all countries. On older logs, in lumber yards and in pulp wood stacks the fungi found are quite different and belong mainly to the Fungi Imperfecti, although some *Ceratocystis* species, e. g. *C. minor*, *C. pilifera* and *C. piceae* may be found even there. Some fungi of the latter group, such as *Tubercullariella ips* and some *Trichosporium* species are also found together with insect infestations.

Physiologically the most thoroughly investigated species are the pathogenic *C. ulmi* and *C. fagacearum*; investigations on the others have dealt chiefly with their vitamin requirements. *C. multiannulata* has been used as a special research fungus by FRIES and collaborators for physiological and genetic investigations. A more detailed review of physiological investigations on these fungi is given in MATHIESEN-KÄÄRIK (1960 a).

Ecological Studies

The material for the studies described here has been collected in different parts of Sweden during the years 1945–1952 with the aid of the Entomological Department of the Forest Research Institute. The insects have been identified by the Entomological Department. The fungal associates of most of insects investigated have not been dealt with previously in other countries.

The fungi were isolated from wood collected at various stages of infestation, from insect galleries and from beetles, larvae and pupae at different stages of development. Older logs and material from saw-mills and timber yards were also investigated. The results are summarised in MATHIESEN 1950 b and MATHIESEN-KÄÄRIK 1953. It was shown that some of the bark beetles were constantly accompanied by blueing fungi and that these fungi could be isolated from tissues surrounding freshly-made galleries even before any stain was visible. Among these bark beetles were *Blastophagus minor*, *Ips acuminatus*, *Hylastes ater*, *Ips sexdentatus* and *Orthotomicus proximus* on pine, *Ips typographus*, *Hylurgops palliatus* and *Hylastes cunicularius* on spruce and *Xyloterus lineatus* on both pine and spruce. The same fungi were always found in fresh infestations of a particular bark beetle and, in some cases they were restricted to a single insect species. Thus *Ceratocystis cana* and *Trichosporium tingens* are the constant associates of *Blastophagus minor* and are only found together with this bark beetle; *C. clavata* and *T. tingens* var. *macrospora* (cf. FRANCKE-GROSMANN 1952) are the associates of *Ips acuminatus* and are only found with it. *C. brunneo-ciliata* and *Tubercullariella ips* have hitherto in Sweden been found only together with *Ips sexdentatus* and *C. ips* has been found only with *Orthotomicus proximus*, but for these last two beetles insufficient material was available to give definite results. On spruce, *Ips typographus* is constantly accompanied by *C. penicillata* and often also by *C. piceae* but these two fungi or eventually several different forms of *C. penicillata* are also commonly found with other bark beetles on spruce, such as *Hylurgops palliatus* and *Hylastes cunicularius*. *Xyloterus lineatus*, on both pine and spruce, is often accompanied by *C. penicillata* and *C. piceae* but it also has a specific associate, a *Monilia*-like brown-coloured fungus. Other bark beetles, for instance *Blastophagus piniperda* and *Pityogenes quadridens* on pine and *P. chalcographus* on spruce were usually accompanied only by yeasts, although occasionally, most in older galleries, they were followed by various blueing and other fungi. *Ceratocystis* species were also found in the galleries of some longicorn beetles, for instance *C. tetropii* with *Tetropium* and *C. olivacea* with *Acanthocinus aedilis*; these fungi, however, cause only slight blueing of the wood. The fungal associate of *Tetropium*, *C. tetropii*, was found in new galleries of the insect in standing trees only in one locality, where it was always found and almost as the only fungus in the larval tunnels and in the surrounding wood. *C. olivacea* at first was found only in the galleries of *Acanthocinus aedilis* but later when examining other materials was often found in spruce stumps which had been attacked by several insects and also in timber yards, on boards. It seems that this fungus prefers rather dry wood, such as in fairly old unbarked logs, stumps and seasoning timber. Of the other longicorn beetles, *Rhagium inquisitor* was usually not accompanied by blueing fungi; with *Monochamus sutor* several different blueing fungi were

sometimes found in older galleries but as the new galleries are mostly without any blueing fungi it seems probable that the fungi in this case had appeared later than the insect, and were disseminated by wind or other insects. *C. minor* is sometimes found with *Pissodes pini*. Since it is one of those blueing fungi which are found sporadically with a number of bark beetles, often in old galleries, and since it is also frequently found in mechanically damaged logs without any insect infestation, its association with *Pissodes pini* cannot be included among the specific associations such as are found between some other bark beetles and blueing fungi. These associations are sometimes particularly specialised, as in the case of *Bl. minor – Ceratocystis cana* and *Ips acuminatus – C. clavata*. Both of these bark beetles live under almost identical conditions underneath the smooth bark of pine top logs and show a similar development, and their distribution areas overlap partly, but each of them is invariably accompanied by its own particular fungal associates. The factors that determine the existence of such very restricted associations are not known. It should be emphasised however that it has been shown that the insects associated with the blueing fungi can develop normally without the fungi (GROSMANN 1930), and also that the ecologically restricted blueing fungi, when grown in pure culture, readily tolerate great variations in the moisture content of the wood or in the nutrient content of the substrate. It seems very probable that there are some specific conditions in the distribution of the fungi which cause such very restricted associations.

Regional differences in the fungal associates of the bark beetles are however known to exist. Nothing is known about the distribution of the associations *Bl. minor – C. cana* and *Ips acuminatus – C. clavata* in other countries. *C. cana* was first described by MÜNCH (1907) as a blueing fungus on pine but since then had been forgotten until it was found to be the constant associate of *Bl. minor* in Sweden (RENNERFELT 1950). *C. ips* has been described as the associate of *Ips pini* in North America, but it has also been found with *Ips sexdentatus* in Germany and Poland and with *Orthotomicus proximus* in Sweden. *Ips sexdentatus* is accompanied in Sweden by a quite different fungus, *C. brunneo-ciliata*. The same applies in the case of Dutch elm disease which in North America is dispersed by insect vectors different from those that spread it in Europe. *C. galeiformis* has been described by BAKSHI (1951 b) from Scotland with *Hylurgops palliatus* on larch; in Sweden it is not infrequently found in spruce stumps in the galleries of *Hylastes cunicularius*. In general, the distribution areas of the blueing fungi are larger than those of their insect vectors but some insect-associated blueing fungi as *C. cana* are known only from restricted areas. Not all blueing fungi of the genus *Ceratocystis* are as strongly bound to fresh bark beetle attack as the species listed above. A number of other *Ceratocystis* species appear in older insect galleries and in the wood around them; they

appear occasionally or sometimes even rather frequently with different bark beetles but as they also frequently appear on mechanically injured or partially barked stems without insect infestations, they are apparently distributed mostly by wind, although some secondary insects might also be involved. The commonest *Ceratocystis* species of this sort are *C. coerulescens*, *C. floccosa*, *C. minor*, *C. minuta*, *C. multiannulata* and *C. pilifera*. Other common airspread blueing fungi such as *Pullularia pullulans* and *Cladosporium herbarum* are also not infrequent in older logs with partially loosened bark. The latter fungi together with *Alternaria*, *Stemphylium*, *Phialophora* spp. and others are the most important blueing fungi in timber yards and saw mills, where their spores are found in the air throughout the year; *Ceratocystis* spores are present only in negligible amounts in the air in these places. (MATHIESEN-KÄÄRIK 1955).

The swarming insects, after hibernation, carry the spores of their fungal associates both externally and internally (MATHIESEN 1950 b), as has already been shown by LEACH *et al.* (1934) for *Ips pini* and *C. ips*. Those bark beetles which are constantly accompanied by blueing fungi (*Blastophagus minor*, *Ips typographus*, *Hylurgops palliatus*) very often carry large numbers of fungus spores but on others (*Bl. piniperda*) fungal spores were found only occasionally (MATHIESEN 1950 b). When the bark beetles start their tunnels under the bark, the blueing fungi begin to grow out almost immediately and they can be isolated from the wood near the galleries already a few days after infestation before any blue stain is visible. This occurs with *Bl. minor* and its fungal associates *C. cana* and *Trichosporium tingens* in Upland already in the last weeks of April and with other bark beetles and fungi somewhat later. From the insect galleries the blueing fungi spread rapidly, radially and longitudinally, the growth rate depending on the species and on the moisture content of the wood. Some fungi such as *C. cana*, *C. clavata*, *C. minor*, *C. penicillata* and *Trichosporium tingens* spread extremely rapidly. Mycelial conidia can very soon after infestation be found in the galleries as sticky glistening masses and are followed by the mucilaginous heads of graphia and other sporophores. Perithecia appear later, during the later larval stages, and usually begin to mature before the larvae begin to pupate, which occurs with *Bl. minor* in Upland in the last weeks of June. The walls of the pupal chambers are usually densely covered with spore masses and the emerging beetles become covered with masses of spores. The mycelial conidia of *Ceratocystis* species were found to be rather short-lived but the more mucilaginous *Graphium* conidia and especially the ascospores, under suitable moisture and temperature conditions, may maintain their viability for several months (MATHIESEN-KÄÄRIK 1960 b). It is thus quite possible that the spores are able to hibernate on the insects and do not need to be preserved in special organs for hibernation, and that they are able to infect the trees in the following spring without any special aid from the insect.

These species of *Ceratocystis*, both the insect-associated and the other species usually are growing in a log during one vegetational period; in the following year there may only occasionally be found species such as *C. minuta*, *C. albida*, *C. minor* and *C. olivacea*. When artificially inoculated in a newly felled log, if the moisture content is suitable, *Ceratocystis* species usually spread rapidly through the whole of the sapwood but they produce much more of all kinds of spores in the cambium, the innermost bark and the 1–2 outermost annular rings of the wood. Thus, although the insect-associated blueing fungi grow rapidly through the sapwood, they sporulate best in the wood and bark layers into which the bark beetles introduce them when making their tunnels.

Taxonomical Studies

From the results described above it is clear that fungi of the genus *Ceratocystis* are prominent among those causing blue stain in association with insect attack on logs. During the above investigations 43 species of blueing fungi were isolated from blue-stained, insect-attacked wood. Thirty species were more or less constantly associated with the insects. Among these there were 19 *Ceratocystis* species and 7 *Graphium*, *Leptographium* and *Scopularia* species, some of which might also be imperfect stages of *Ceratocystis*; only 4 species of the insect-associated blueing fungi belonged to other Fungi Imperfecti. The remaining 13 species which were found only occasionally with insects are fungi with more or less coloured mycelia belonging to different genera of Fungi Imperfecti. They have been grouped as "air-spread blueing fungi" (Mathiesen 1950 b, Mathiesen-Käärik 1953), some of them being fungi whose spores are frequent almost everywhere in the air (Mathiesen-Käärik 1955). Of the air-spread blueing fungi, *Pullularia pullulans* is often listed in the tables but this is mainly due to its rather common occurrence in old samples.

In previous papers the generic name *Ophiostoma* H. & P. Syd. has been used as suggested by Melin & Nannfelt (1934). The history of this genus and of the older *Ceratostomella* Sacc., which until very recently was still accepted in America, has been discussed previously (Mathiesen 1951). Further division of the genus into new ones based mainly on divergent imperfect stages, such as *Endoconidiophora* Münch and *Grosmannia* G. Goid. is not well founded, as their perfect stages are inseparable and forms intermediate between the different conidial types occur (Mathiesen 1951). In his monograph on the genus *Ceratocystis* Hunt (1956) has taken the same view. Bakshi (1950) revived the generic name *Ceratocystis* Ell. & Halst. for species of *Ceratostomella* with irregular evanescent asci and as is discussed by Hunt (1956), there are good grounds for accepting this generic name as valid.

Of the 19 *Ceratocystis* species listed above, 6 were recorded for the first time in Sweden and 6 were new species. The new species have been described as they appeared in pure cultures and cultures of these and some other more uncommon species have been deposited at CBS, Baarn. Cultures and herbarium material were also sent to Dr. JOHN HUNT, Syracuse University, who has incorporated the herbarium material in his collections. Dr. HUNT has accepted the proposed new species in his monograph.

The 6 *Ceratocystis* species new for Sweden were: *C. cana* (Münch) C. Moreau, *C. ips* (Rumb.) C. Moreau, *C. galeiformis* Bakshi, *C. minuta* (Siem.) Hunt, *C. penicillata* (Grosm.) C. Moreau and *C. polonicum* (Siem.) C. Moreau.

The new species were: *C. albida* (Math.-K.) Hunt, *C. brunneociliata* (Math.-K.) Hunt, *C. clavata* (Math.) Hunt, *C. floccosa* (Math.) Hunt, *C. olivacea* (Math.) Hunt, and *C. tetropii* (Math.) Hunt, described in MATHIESEN 1951 and MATHIESEN-KÄÄRIK 1953. As the published work on the Swedish *Ceratocystis* species is spread over several papers, it seemed to be of value to include in this paper a review of the *Ceratocystis* species found in Sweden together with references to Scandinavian papers in which they are discussed more thoroughly and with some short notes on their occurrence and taxonomy.

Descriptions of all these species and references to their original descriptions are given in HUNT's monograph and have not been cited here. Some synonyms are listed, as the fungi in question have in the past been generally known under the names *Ophiostoma* and *Ceratostomella* and as some well known specific names now become only synonyms of less known names.

HUNT's division of the genus into sections according to the imperfect stages seems practical and has been followed below but further subdivision into groups and subgroups is modified according to Swedish material with the aim to enable a preliminary identification of the species.

Swedish Ceratocystis Species

Section 1. Species with an endoconidial imperfect stage.

Ceratocystis coerulescens (Münch) Bakshi.

Endoconidiophora coerulescens Münch. MÜNCH 1907, LAGERBERG, LUNDBERG & MELIN 1927.

Occurrence[1]): Bogesund, Enebyberg, Åkersberga, Skutskär (Upl.); Hamra, Mora, Siljansfors (Dlr.); Hofors (Gstr.); Gällivare (Lpl.). Not rare, but occurring more sporadically than for instance *C. pilifera*. Mostly on spruce, but found also on pine. Not associated with insects. The Swedish material is very

1) Abbreviations of Swedish provinces: Skåne (Sk.), Blekinge (Bl.), Halland (Hl.). . . .

constant in its morphological characters. Easily recognised in culture and on wood by its distinctive odour.

Section 2. Species with Leptographium or Graphium imperfect stage.

I. With Leptographium conidial stage.

Ceratocystis penicillata (Grosm.) C. Moreau.

Ophiostoma penicillatum (Grosm.) Siem. Mathiesen 1950 b.

Occurrence: Dalby, Krageholm (Sk.); Lister (Bl.); Tönnersjöheden (Hl.); Bläsinge, N. Möckleby (Öl.); Gustav Adolf (Vg.); Högby mo, Regna (Ög.); Fagersjö, Handen, Järna, Kagghamra, Katrineholm (Sdl.); Lilljanskogen, Gålö, Stavsnäs (Sthlm.); Bogesund, Dimbo, Gardvik, Enebyberg, Kungsängen, Lovö, Rolen, Skutskär, Sundby, Åkersberga (Upl.); Braskfall, Lotorp, Rattsjöberg, Sunnemo, Sunnerbo, Västrottna (Vrml.); Mora, Njupeskär, Siljansfors, Älvdalen (Dlr.); Hofors, Ockelbo (Gstr.); Torsboda (Mpd.); Glöte (Hrj.); Strömsund, Ulriksfors (Jtl.); Hällnäs, Robertsfors, Svartberget, Vindeln (Vb.); Murjek, Gällivare (Lpl.).

Very common in the whole country on spruce, but occurs also on pine. This fungus is very variable both in perithecial characters and in the imperfect stage and perhaps some forms or even species may be separated further. In Mathiesen 1950 b, descriptions were given of several forms but as the characters which seemed to be distinctive in freshly isolated cultures gradually change during further cultivation, it has not been possible in later investigations to separate good forms with constant characters. The typical form as described by Grosmann (1932) with not very large, rather long- and stout-necked perithecia, is associated with *Ips typographus*. With other bark beetles both on spruce and pine there are found forms which are quite distinct in fresh isolations in their imperfect stage but which did not show any real and constant differences in the perithecial stage. There exists, however, one type, isolated from logs and stumps of both pine and spruce, which has somewhat larger perithecia, c. 250 μ in diam., with shorter necks, usually the same length or shorter than the diameter of the perithecium, generally producing among the normal perithecia others, occasionally very numerous, which have no necks and discharge the mature spores through an irregular rupture in the perithecial wall. A new species and genus, *Europhium trinacriforme*, has been described by Parker (1957), which differs from *C. penicillata* mainly by its neckless perithecia. The Swedish form of *C. penicillata* described above seems to be intermediate between the typical *C. penicillata* and *Europhium trinacriforme*. It was also physiologically different from typical *C. penicillata*. Its imperfect stage could not, however, be distinguished from that of typical *C. penicillata*.

Ceratocystis polonicum (Siem.) C. Moreau.

Ophiostoma polonicum Siem. MATHIESEN 1950 b, 1951.

Occurrence: Lotorp (Vrml.); Hofors (Gstr.); Vindeln (Vb.).

Found occasionally on spruce logs in the old galleries of bark beetles together with other fungi and once in a spruce stump. Resembles *C. penicillata* in cultural characters but differs in having perithecia with long slender necks and ostiolar cilia and in producing only sparse conidia but very numerous perithecia.

II. With Graphium conidal stage.

A. Perithecia with short necks, up to 400 μ long

C. olivacea (Math.) Hunt.

Ophiostoma olivaceum Math. MATHIESEN 1951.

Occurrence: Tönnersjöheden (Hl.); Järna (Sdl.); Bogesund, Skutskär (Upl.); Braskfall, Deje, Lotorp, Rattsjöberg, Sunnemo, Vassgårda, Västrottna, Uddeholm (Vrml.); Hofors (Gstr.); Enaforsholm (Jtl.), Hällnäs, Vindeln (Vb.).

Found at first with the longicorn beetle *Acanthocinus aedilis* in pine and spruce logs but later also in lumber yards and often in both pine and spruce stumps. It can apparently grow even on rather dry wood. In cultural appearance it rather resembles *C. galeiformis* but the perithecia of *C. olivacea* differ from most *Ceratocystis* species in being small with short, stout necks with long ostiolar cilia. Produces regular rather short-stalked graphia in the imperfect stage but sometimes small irregular graphia of the *C. ips* type can be seen. Appears often in the conidial stage and produces perithecia very slowly.

B. Perithecia with longer necks, usually over 500 μ.

a. With regular *Graphium* conidiophores.

C. piceae (Münch) Bakshi.

Ophiostoma piceae (Münch) H. & P. Syd. LAGERBERG, LUNDBERG & MELIN 1927, MATHIESEN 1950 b.

Occurrence: Dalby, Krageholm, Margaretetorp (Sk.); Tönnersjöheden (Hl.); Asarum (Bl.); Växjö (Sm.); Bläsinge (Öl.); Gustaf Adolf (Vg.); Högby mo, Regna (Ög.); Fagersjö, Handen, Kagghamra, Katrineholm, Järna, (Sdl.); Lilljanskogen (Sthlm.); Bogesund, Brobacka, Enebyberg, Kungsängen, Lovö,

Skutskär, Åkersberga (Upl.); Björneborg, Braskfall, Deje, Lotorp, Kollerud, Rattsjöberg, Sunne, Sunnemo, Vänersvik, Västrottna, Uddeholm (Vrml.); Hofors (Gstr.); Hamra, Mora, Njupeskär, Siljansfors, Älvdalen (Dlr.); Torsboda (Mpd.); Glöte (Hrj.); Ulriksfors (Jtl.); Hällnäs, Vindeln (Vb.); Murjek (Lpl.).

Very common over the whole country on spruce, sporadically on pine. It is often found with bark beetle infestations even at the beginning of the attack but also often without insect infestations. Very constant morphologically and produces perithecia readily. Sometimes found on wood only in the *Graphium* stage which is macroscopically very similar to that of *C. cana* but differs in having small ovoid-ellipsoid conidia. *C. floccosa* also resembles *C. piceae* but differs in some perithecial and *Graphium* characters. Two very similar species found on deciduous trees, *C. quercus* and *C. fagi* are regarded as synonyms of *C. piceae* in HUNT's monograph.

Ceratocystis cana (Münch) C. Moreau.

Ophiostoma cana (Münch) H. & P. Syd. MÜNCH 1907, MATHIESEN 1950 b.

Occurrence: Tönnershöheden (Hl.); Asarum (Bl.); Växjö (Sm.); N. Möckleby (Öl.); Regna, Högby mo (Ög.); Järna, Fagersjö, Handen, Katrineholm (Sdl.); Bogesund, Enebyberg, Dimbo, Lovö, Rolen, Skutskär, Stavsnäs, Täljö, Älvkarleby, Åkersberga, Ålavan (Upl.); Hofors, Ockelbo (Gstr.); Hamra, Mora, Siljansfors (Dlr.); Rönnåsen (Hls.); Bispgården, Sollefteå, Ulfsta (Ång.); Hällnäs (Vb.).

Very common on pine top logs from South Sweden to Västerbotten; found only with *Blastophagus minor*. Exists usually in the *Graphium* stage which in habit is very similar to *C. piceae* but differs in its large round conidia. The perithecial stage is also rather similar to that of *C. piceae*. Physiologically entirely different from *C. piceae* strains.

Ceratocystis floccosa (Math.) Hunt.

Ophiostoma floccosum Math. MATHIESEN 1951.

Occurrence: Bogesund, Enebyberg (Upl.); Hofors (Gstr.); Hamra (Dlr.); Hällnäs (Vb.).

Occurs sporadically on both pine and spruce, mostly with mechanically injured bark, not associated with insects. Mostly in *Graphium* stage, perithecia not very numerous, produced slowly. The conidial stage differs from

that of *C. piceae* in the flat-topped graphia with yellowish heads; perithecia smaller and with longer necks and shorter ostiolar hyphae than these of *C. piceae*. Differs physiologically from *C. piceae*.

Ceratocystis ulmi (Buism.) C. Moreau.

Ophiostoma ulmi (Buism.) Nannf.

Occurrence: Göteborg; on the East coast between Kalmar and Stockholm, e. g. Kalmar, Borgholm, Ryssby, Blomstermåla, Oskarshamn, Gamleby, Gusum, Norrköping, Trosa, Södertälje, Stockholm and surroundings, and in Upland e. g. Uppsala, Salsta, Brunna, Sigtuna, Öregrund.

Found on elms; the only parasitic species of *Ceratocystis* occurring in Sweden. Perithecia are rarely found in nature. Insect vectors in Sweden are *Scolytus* spp.

b. With claviform *Graphium* coremia.

Ceratocystis brunneo-ciliata (Math.-K.) Hunt.

Ophiostoma brunneo-ciliata Math.-K. Mathiesen-Käärik 1953.

Occurrence: Njupeskär (Dlr.); Korskrogen (Hls.); Murjek (Lpl.); Hamptjärn (Nb.).

Found infrequently in the northern parts of the country, on pine. Associated with *Ips sexdentatus*. Very fast growing, with very dark mycelium, large, very long-necked (up to 1400 μ) perithecia with brown corkscrew-like ostiolar cilia. Large *Graphium* coremia with white clavate heads. Resembles *C. clavata* but differs in its much larger perithecia and graphia. Physiologically different from *C. clavata*.

Ceratocystis clavata (Math.) Hunt.

Ophiostoma clavatum Math. Mathiesen 1951

Occurrence: Järna (Sdl.); Skutskär (Upl.); Hofors (Gstr.); Hamra, Idre, Mora, Njupeskär, Älvdalen (Dlr.); Ulriksfors, Östersund (Jtl.); Hällnäs, Långsele, Robertsfors, Svartberget, Vindeln (Vb.); Akkavare, Murjek, Gällivare (Lpl.).

On pine top logs, associated with *Ips acuminatus*, common in the northern parts of Sweden, from Dalarna to Lapland. Resembles a dwarf strain of *C. brunneo-ciliata*. Comparatively small perithecia with necks 400–800 μ, corkscrew-like ostiolar cilia. Graphia claviform, up to 500 μ high. Rather constant in morphological characters.

c. Conidial stage irregular, transitional forms between *Leptographium* and *Graphium* coremia always present.

Ceratocystis ips (Rumb.) C. Moreau.

Ophiostoma ips (Rumb.) Nannf. MATHIESEN-KÄÄRIK, 1953.

Occurrence: Järna (Sdl.); Njupeskär (Dlr.); Korskrogen (Hls.); Murjek (Lpl.)

Found sporadically in the northern parts of Sweden on pine logs, associated with *Orthotomicus proximus*. Compared with American forms the Swedish form of *C. ips* produces rather large and rather long-necked perithecia. In the imperfect stage it produces conidiophores which resemble small graphia but are mostly intermediate between *Leptographium* and *Graphium*. Typical *Leptographium* and *Graphium* coremia are also found but the latter are always rather small and with slender stalks.

Ceratocystis galeiformis Bakshi.

MATHIESEN-KÄÄRIK 1953.

Occurrence: Mora (Dlr.); Glöte (Hrj.); Hällnäs, Vindeln (Vb.).

Found hitherto rarely in the northern parts of Sweden, in spruce stumps, appears to be associated with *Hylastes cunicularius*. The cultural appearance resembles that of *C. olivacea* but its rather large, long-necked perithecia without ostiolar cilia are entirely different. With *Graphium* imperfect stage but produces numerous graphia as well as a complete range of intermediates between *Leptographium* and *Graphium*. Rather similar to *C. olivacea* in its vitamin and carbohydrate requirements; differs in its nitrogen requirements.

Section 3. Species with mycelial conidia only.

I. Perithecia small, 70–120 (–140) μ in diam.

A. With short necks, not longer than the diameter of the perithecia.

Ceratocystis minor (Hedgc.) Hunt.

Ceratostomella minor Hedgc.
Ophiostoma pini (Münch) H. & P. Syd. LAGERBERG, LUNDBERG & MELIN 1927, MATHIESEN 1950 b.

Occurrence: Dalby, Krageholm (Sk.); Tönnersjöheden (Hl.); Asarum, Lister (Bl.); Växjö (Sm.); Bläsinge, N. Möckleby (Öl.); Högby mo, Regna (Ög.); Gustav Adolf (Vg.); Fagersjö, Handen, Järna, Kagghamra, Katrineholm (Sdl.); Lilljanskogen, Stavsnäs (Sthlm.); Bogesund, Brobacka, Dimbo, Enebyberg, Gardvik, Lovö, Skutskär, Täljö, Åkersberga, Älvkarleby (Upl.); Hofors, Ockelbo (Gstr.); Braskfall, Rattsjöberg, Sunnemo, Sunnerbo, Väst-

rottna (Vrml.); By, Hamra, Idre, Mora, Njupeskär, Siljansfors, Älvdalen (Dlr.); Korskrogen (Hls.); Glöte, Sveg (Hrj.); Strömsund, Ulriksfors (Jtl.); Hällnäs, Långsele, Robertsfors, Svartberget, Vindeln (Vb.); Arvidsjaur, Murjek, Gällivare (Lpl.).

Very common all over the country on pine. Found occasionally with a number of bark beetles but usually in old galleries. Very common even in logs without insect infestations, in lumber yards and in pine stumps. This species seems to have a very wide range in its moisture requirements. It is easy to identify by its very numerous, very small, short, stout-necked perithecia, without ostiolar cilia.

Ceratocystis minuta (Siem.) Hunt.

Ophiostoma minutum Siem. Mathiesen 1951.

Occurrence: Järna (Sdl.); Bogesund (Upl.); Hofors (Gstr.); Hamra (Dlr.); Ulriksfors (Jtl.).

Found rarely, mostly on pine but sometimes on spruce, in old galleries of different bark beetles together with other fungi. Not associated with any special insect. A very slow-growing almost colourless fungus with not very numerous, small perithecia with short stout necks and ostiolar cilia. Ascospores filiform, discharged usually not in drops as in other *Ceratocystis* species but in long threads.

Ceratocystis albida (Math.-K.) Hunt.

Ophiostoma albidum Math.-K. Mathiesen-Käärik 1953.

Occurrence: Järna (Sdl.); Åkersberga (Upl.); Mora, Älvdalen (Dlr.).

Found sporadically on pine and spruce in the old galleries of different bark beetles; prefers rather moist wood. An almost colourless slowly growing fungus with typical small and rather long-necked perithecia, necks 300–400–500 μ long. Resembles *C. stenoceras* but is distinguished by its slower growth and much smaller perithecia.

II. Perithecia about 150 μ in diam. or larger, with long necks, always longer than the diameter of the perithecia.

Ceratocystis pilifera (Fries) C. Moreau.

Ceratostomella pilifera (Fries) Wint.

Ophiostoma coeruleum (Münch) H. & P. Syd. Lagerberg, Lundberg & Melin 1927, Mathiesen 1950 b.

Occurrence: Tönnersjöheden (Hl.); Bläsinge (Öl.); Högby mo (Ög.); Handen, Järna, Kagghamra (Sdl.); Bogesund, Dimbo, Enebyberg, Skutskär, Åkersberga

(Upl.); Hofors (Gstr.); Braskfall (Vrml.); Torsboda (Mpd.); Hällnäs (Vb.); Gällivare (Lpl.).

Occurs sporadically with insect infestations on pine, but is rather common in old logs, in logs with mechanically injured bark and in timber yards. The length of the necks may be rather variable in nature, sometimes reaching that of *C. pluriannulata* (3000 μ), but is shorter and more constant in culture. In other respects rather constant in appearance. Cultures are inclined to sectoring and usually degenerate rather soon.

Ceratocystis pluriannulata (Hedgc.) C. Moreau.

> *Ophiostoma pluriannulatum* (Hedgc.) H. & P. Syd. Lagerberg, Lundberg & Melin 1927, Mathiesen 1950 b.

Occurrence: Järna (Sdl.); Enebyberg, Skutskär (Upl.); Uddeholm (Vrml.); Hofors (Gstr.); Korskrogen (Hls.); Vindeln (Vb.).

Found only occasionally, mostly on pine, once on spruce, in old galleries of different bark beetles together with other fungi. Mycelium almost colourless, growing rather slowly, perithecia with very long and slender necks (about 3000 μ), with several whorls of cilia. Degenerates rather soon in culture.

Ceratocystis tetropii (Math.) Hunt.

> *Ophiostoma tetropii* Math. Mathiesen 1951.

Occurrence: Hofors (Gstr.)

This species has been found only in one locality, where it frequently occurs in standing spruce trees at the base of the trunk in galleries of *Tetropium* sp. This homothallic species is very typical in appearance with pale yellow-brown mycelium, large perithecia covered with brown hyphal hairs and with long sharply-pointed ostiolar hyphae. This fungus has been maintained in culture without altering for about ten years and still produces numerous typical perithecia.

C. stenoceras (Robak) Hunt.

> *Ceratostomella stenoceras* Robak. Robak 1932.

Occurrence: Högby mo (Ög.); Järna (Sdl.); Skutskär (Upl.); Vindeln (Vb.).

Found sporadically on both spruce and pine without insect infestations. Resembles *C. albida* but has larger perithecia with longer necks. The Swedish isolations agree well with Robak's description. Hunt, however, in his monograph includes this species among the species dubiae as he found the only available cultures, those from Baarn, to be atypical. Differs physiologically from *C. albida*.

Physiological Studies

Some physiological experiments have been carried out on the fungus material described above (MATHIESEN 1950 a, MATHIESEN-KÄÄRIK 1960 a). The purpose of these experiments was principally to find conditions favourable for growth and for the production of different types of spores on synthetic media and to ascertain if there were any differences between fungi belonging to different ecological groups.

Growth.

It was found that almost all the *Ceratocystis* species tested were vitamin-heterotrophic, in agreement with the results of investigations by other authors on *Ceratocystis*. The insect-associated Fungi Imperfecti were also vitamin-heterotrophic but the common airspread blueing fungi from the same group were vitamin-autotrophic. The vitamins for which the fungi tested were partially or totally deficient were thiamine, pyridoxine, and biotin, either singly or in combinations. Frequently the fungi were deficient for two vitamins, all possible combinations between the three being found. Some fungi, however, were found to be vitamin-autotrophic, among them *C. brunneo-ciliata* which is undoubtedly an insect-associated fungus. The degree of heterotrophy apparently changed sometimes when a fungus was cultivated for a long period. Different strains of the same species were not absolutely identical in their vitamin requirements; usually they were deficient for the same vitamins but the degree of deficiency varied. Strains of the morphologically unstable species *C. ulmi* and *C. penicillata* were found which were vitamin-autotrophic though these species are usually vitamin-heterotrophic. These two deviating vitamin-autotrophic strains also showed morphological differences. However, the material available was not sufficient to show whether deviant strains of this kind were of more general occurrence.

There were no differences between the vitamin requirements of fungi growing on pine or spruce, or belonging to different ecological groups. Two fungi growing simultaneously in the same place may have quite different vitamin requirements. Aspartic acid, oleic acid and Tween 80 (an oleic acid derivative) all had a growth-promoting effect on biotin-deficient fungi but the effect varied in different fungus strains; aspartic acid gave the best results with some fungi, Tween 80 with others. In no case, however, were the results entirely comparable with those obtained with biotin. No vitamins other than those listed above were required for the growth of these fungi; the addition of malt and yeast extracts as vitamin sources to synthetic media containing glucose and asparagine often had an effect inferior to that obtained by addition of the pure vitamins.

Table 1. Growth of *Ceratocy*

Fungus	Ecological group	glucose, fructose, manose, galac-tose	cello-biose	xylose	maltose	pectin	glycerol	rham-nose	oi-c ac
C. brunneo-ciliata	I. Primary	+++	+++	+++	+++	+++	+++	+++	
C. cana	blueing fungi	+++	+++	+++	+++	+++	++	+++	++
C. clavata	on pine	+++	+++	+++	+++	++	+++	+++	++
C. penicillata	II. – do. –	+++	+++	+++	+++	+++	+++	+++	++
C. piceae	on spruce	+++	+++	+++	+++	+++	+++	+++	++
C. tetropii		+++	+++	+++	+++	+++	+++	+++	++
C. ulmi	III. Primary fungi	+++	+++	+++	+++	+++	+++	+++	++
C. 367	on deciduous	+++	+++	+++	+++	+++	+++	++	++
C. 693	trees	+++	+++	+++	+++	+	+++	+++	+
C. galeiformis	IV. Secondary	+++	+++	++	++	+++	+++	+++	+
C. minor	blueing fungi,	+++	+++	+++	+++	+++	+++	+++	++
C. olivacea	insect associated	+++	+++	+++	+	+++	+++	++	+
C. albida	V. – do. –	+++	+++	+++	+++	+++	++	+++	++
C. coerulescens	air-distributed	+++	+++	+	+++	+++	+	+	+
C. floccosa		+++	++	+++	+++		+++	+++	
Scopularia phycomyces		+++	+++	+++	+++	+++	+++	++	++

+++ good growth ++ fair growth + poor growth — no growth no sign : no experiment

The only exception was *Trichosporium tingens*, a closely insect-associated fungus, which seems to require some other growth factor.

Tests were made of the growth of the blueing fungi on different carbon sources. The results are summarised in Table 1, in which the fungi are listed according to their substrates and the sugars according to their utilisability. If more fungi and carbohydrates were tested, the picture might change somewhat; however, certain differences can be seen in the utilisability of a series of carbon sources by fungi of different ecological groups. The largest number of different carbon compounds is utilised by the primary fungi initiating the infection on deciduous trees, especially by the parasitic *C. ulmi*. After this group come the primary blueing fungi on spruce and then the primary blueing fungi on pine. The more secondary blueing fungi could utilise fewer compounds, although *C. albida* and *C. floccosa* behaved more like the primary fungi. All the fungi investigated could readily utilise the hexose sugars glucose, fructose, mannose and galactose, and also the disaccharide cellobiose, and with some exceptions also xylose, maltose, pectin, glycerol, rhamnose and oleic acid. With arabinose, sorbitol, mannitol and inositol the results were more variable. The di- and polysaccharides, and ribose, sorbose, erythritol and malic acid were often poor carbon sources, especially for the secondary blueing fungi, though the primary fungi were more or less able to use them. Sucrose is a good carbon source for

pecies on different carbon sources.

sorbitol	inositol	arabinose	mannitol	starch	sucrose	sorbose	erythritol	malic acid	lactose	raffinose	inulin	ribose	cellulose
+++	+	+	+++	+++	+	++	+++	++	+	+	+	+	+
++	++	++	+++	+++	++	+	+++	++	+++	++	+++	+	+
+++	+	+++	+++	++	+	+	++	++	++	+	+	+++	++
+++	+++	+++	++	++	+++	++	++	+	+	+	+++	++	+
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+++	+++	+++	+++	+++	+++	+++	+	++	+++	+++	++	+	+
+++	+++	+++	+++	++	+++	++	++	++	+++	++	+	+	+
+++	++	+++	+++	+	+++	+	++	+	+++	+++	+	+	+
+	+++	+++	+	+	+	++	+	+	+	+	+	+	+
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++	+++	+	+	++	+++	++	++	++	+	+++	++	+	+
++	+	+++	++	+++	+++	+	+	–	+++	+	++	+	+
++	++	+++	+++	+++	+++	+++	++		++	++	+	++	++
++	++	++	++	++	+	++	++	+	+	+	+	+	+

the primary fungi on deciduous trees and on spruce but the primary fungi on pine and a group of secondary fungi are unable to utilise it. Raffinose and lactose are utilised by the fungi from deciduous trees and by some occasional species from the other groups; starch seems to be utilised best by the pine primary fungi and inulin by the spruce fungi. Of the sugar alcohols, inositol is utilised best by the secondary fungi; for the others it is often a poor substrate but individual variations are of course great. All the alcohols were utilised well by the spruce fungi and, except for erythritol, also by the deciduous tree fungi. For the pine fungi erythritol, sorbitol and mannitol are good carbon sources, but not inositol. Ribose and cellulose are utilised only by a few of these fungi. Thus, it can be seen that there are differences in the utilisation of carbon sources by fungi of different ecological groups and from different host trees, the primary fungi being able to utilise a greater number of compounds, often even di- and polysaccharides, while the secondary fungi have difficulties with the latter. An exception is the insect-associated primary blueing fungus on pine, *Trichosporium tingens*, which grows only on a few carbon sources.

Tests were also made of the growth of blueing fungi on various nitrogen sources, including calcium nitrate, ammonium sulphate, ammonium tartrate, asparagine, glutamine and alanine. Again, *Trichosporium tingens* was unable to grow on these common nitrogen sources in a purely synthetic medium. *C. ips*

was the only other fungus which could not utilise ammonium sulphate; apart from these two all the fungi grew on all the nitrogen compounds tested except nitrate. Of the 23 fungi tested only 9 could utilise nitrate, most of them secondary blueing fungi.

Sporulation

The production of mycelial conidia under different experimental conditions was very variable. Usually, if good mycelial growth occurred, the production of mycelial conidia was also good. If growth conditions were less favourable, there was sometimes only mycelial growth but often on such media the production of mycelial conidia was stimulated. This was especially significant with media, where growth was inhibited by the absence of some necessary vitamins. No special vitamin was involved in the stimulation or inhibition of the production of mycelial conidia but in general any disturbance in the vitamin supply resulted in alterations of the balance between mycelial and conidial growth. On carbon sources unsatisfactory for growth, the production of mycelial conidia was sometimes inhibited, but some, especially inositol, stimulated the production of conidia. With citrate as carbon source many of the *Ceratocystis* species produced only conidial growth.

The production of *Graphium* or *Leptographium* sporophores was not stimulated on the media which gave maximum production of mycelial conidia. Coremia were never found on media which were deficient for some vitamin required by the fungus for growth. When a complete set of vitamins was added, sporophores were produced very numerously on a variety of media. The dependence of asexual sporophore production on the vitamin supply was thus the same as for the production of perithecia. Some nutrient media with various carbon and nitrogen sources were especially stimulatory for just this type of spore but the results with different fungi were very variable. No particular compound had a general stimulating effect on the production of sporophores in all *Ceratocystis* species or in all the species in any morphological or ecological group. The concentration of the nutrients was a decisive factor in sporophore production. The optimal production of sporophores occurred at rather high carbohydrate and relatively high nitrogen concentrations with a lower carbon-nitrogen ratio than for the production of perithecia. Still higher nitrogen concentrations limited the production of sporophores. At low carbohydrate concentrations no sporophores were produced but at low nitrogen concentration the production of sporophores continued; although formed in the same numbers their size was diminished.

Perithecia were produced if the fungi were supplied with adequate amounts of the right nutrients and all the vitamins which were required for maximum

growth. No further vitamins were required. In the case of *C. tetropii* it was shown that the vitamins were not needed in greater concentrations for the production of perithecia than for maximum mycelial growth under the experimental conditions. The amount of vitamins needed both for growth and for production of perithecia was dependent on the nutrient concentrations in the medium.

Some carbon and nitrogen sources were better than others for the production of perithecia; in general they were not the same compounds that gave the best results for sporophore production. The production of perithecia was not dependent on the amount of mycelium produced with different carbon and nitrogen sources. The intermediate metabolism in the breakdown and assimilation of the different carbon and nitrogen compounds seems to be decisive for perithecial production with different nutrients. The concentration of nutrients is of great importance. Perithecia are only produced when the available carbon concentration is rather high but not too high and the nitrogen concentration is low – for some fungi almost 0. The C/N ratio for optimal production of perithecia is higher than for the production of asexual coremia. The C/N ratio for the production of perithecia, was not, however, the same for all the fungi. Some *Ceratocystis* species produce perithecia readily only if the nitrogen content of the medium is near 0; others prefer somewhat higher nitrogen concentrations. When the carbon source added is poorly utilised by the fungi, the lowest nitrogen concentrations are generally preferable for the production of perithecia; with readily utilised carbon sources perithecia may be produced even at higher nitrogen levels. The *Ceratocystis* species produced perithecia readily even on nitrogen sources which were poorly utilised, especially in the case of nitrate nitrogen. Thus, it is not the presence or absence of nitrogen compounds or of some specific nitrogen compound in the medium that is decisive for the production of perithecia but the nitrogen starvation of the cells. Obviously some changes in cell metabolism which are induced by the nitrogen deficiency must occur before the production of perithecia is initiated.

The production of perithecia generally but not invariably parallels pigment production. The pigment production must be regarded as an expression of normal cell functioning and it seems that in the case of *Ceratocystis* there is no true causality in the relation between pigment and perithecium production.

Discussion

In nature, the production of mycelial conidia and sporophores begins immediately after the wood is infected in spring. Perithecia are found in late spring and early summer in the insect galleries and are usually produced in great numbers at the pupal time. While the perithecia are appearing no more graphia

are produced. As the nitrogen content of the wood is very low, the production of perithecia might well be induced by the exhaustion of nitrogen in the wood nearest the insect galleries. Even in wood which is not infested by insects, the greatest numbers of spores are found in those layers where insect tunnels occur when present – in the cambium and the youngest layers of phloem and wood. Obviously these layers must contain some nutrients that stimulate growth and sporulation of the blueing fungi. Little is known about the different compounds which may occur in these layers. Tests were made of the effect on growth and sporulation of glutamine which is frequently found to be the dominant nitrogen compound in young dividing cells but in pure cultures no differences were found between glutamine and asparagine, the main nitrogen constituent of spruce wood. In any case the layers preferred by *Ceratocystis* are also those in which the bark beetles live but this is true for all *Ceratocystis*, and no specific differentiation was observed.

The ascospores are the most long-lived of the various types of *Ceratocystis* spores and seem in most cases to play the most important role in the dispersal of the species. The ascospores can be found in great masses when the new beetles are emerging. The timing of the development of the fungi is thus well adapted to that of their insect vectors. However, the production of ascospores is apparently not always important for the dispersal of a species, because insect-associated *Ceratocystis* species such as *C. ulmi* and *C. cana* normally only produce *Graphium* conidia. The *Graphium* conidia of *C. cana* were found to be more long-lived than those of some other *Ceratocystis* species, which usually produce ascospores, for instance *O. piceae*. In such cases the long-lived *Graphium* conidia have taken over the role of ascospores.

In their nutritional physiology the ecologically restricted blueing fungi show no special differentiation, but all the fungi investigated differ somewhat in their nutritional requirements. Generally all the *Ceratocystis* species are deficient for several vitamins; this is also the case with the insect-associated Fungi Imperfecti, but the common air-spread blueing fungi are vitamin-autotrophic. The same relation has been found in yeasts. The more primary *Ceratocystis* species are able to utilise the largest number of the carbohydrates tested, especially the di- and polysacharides, and thus show a wider enzymatic capacity and perhaps a greater compatibility than the more secondary blueing fungi. These most probably are able to colonize only wood which has been occupied by other organisms. The only insect-associated blueing fungus with quite different nutritional requirements is *Trichosporium tingens*, a species which also ecologically has been regarded as different from *Ceratocystis*, and has been classified by FRANCKE-GROSMANN (1952) as an ambrosia fungus, i. e. a fungus which is required by the insects for their nutrition. It has been shown that this fungus is spread in a special way which has not hitherto been found in *Cerato-*

cystis. Even though no major physiological differences were found between the fungi associated with different insects and the non-associated species, every fungus has its special nutritional and moisture requirements and development time (MATHIESEN-KÄÄRIK 1953 and unpublished experiments). All these factors together might produce optimal conditions for some particular fungus or fungi in the galleries of a specific bark beetle; other fungi may occasionally be introduced into the galleries of this insect, but are not compatible for any longer time and would disappear in succeeding insect generations. This would fully explain the existence of such not very specialised associations which are found between some wide-spread blueing fungi and a number of different but ecologically similar bark beetles. On the other hand, this does not fully explain the existence of the very specific associations, as between *Bl. minor* and *C. cana*, for which some other hitherto unknown factors must be decisive, most likely concerning their dispersal biology.

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